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RESEARCH PROJECT INITIATION

Date: October 11, 1974

Project Title: Evaluation of the Efficacy of Chlorination of Hospital Wastes

Project No: E-20-662

Principal Investigator Dr. A. W. Hoadley

Sponsor: Ga. Department of Natural Resources

Agreement Period: From 7/1/74 Until 10/31/74

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Assigned to: Civil Engineering

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RESEARCH PROJECT TERMINATION

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Project Title Evaluation of the Efficacy of Chlorination of Hospital Wastes

Project No: E-20-662

Principal Investigator: Dr. A. W. Hoadley

Sponsor: Ga. Department of Natural Resources

Effective Termination Date: 10/31/74

Clearance of Accounting Charges: 10/31/74

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E-20-662

Evaluation of the Efficacy of Chlorination of Hospital Wastes

A Report to the
Environmental Protection Division
Georgia Department of Natural Resources
270 Washington St., S.W.
Atlanta, Georgia 30334

Prepared by
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October, 1974

Evaluation of the Efficacy of
Chlorination of Hospital Wastes

by Alfred W. Hoadley

Since April, 1974, samples have been obtained from Olley Creek in Cobb County, Georgia to determine the effects of hospital wastes on bacterial water quality. Populations of both Pseudomonas aeruginosa and fecal coliforms have been determined in stream water samples. In July, 1974, the studies were extended, with partial support of the Environmental Protection Division of the Georgia Department of Natural Resources, to determine the effects of treatment, and in particular, the effects of chlorination, on populations of P. aeruginosa, fecal coliforms, and antibiotic resistant coliforms in wastes discharged to the Creek from the Cobb General Waste Treatment Plant.

BACKGROUND OF THE STUDY

PSEUDOMONAS AERUGINOSA AND ITS SIGNIFICANCE. Pseudomonas aeruginosa is a highly versatile bacterial species which causes a wide variety of diseases in plants, animals, and man, including ear infections among swimmers and water-borne mastitis in cattle. It is a pathogen of increasing concern to man because it is resistant to most antibiotics and frequently invades patients receiving antibiotic therapy. It is a major problem in cancer wards, burn wards, and newborn nurseries of hospitals where patients have low resistance to disease. It normally is susceptible to carbenicillin and gentamicin, but resistant strains have been isolated recently from leukemia patients receiving intensive therapy.

While wound infections and carriers are often the sources of the organisms causing outbreaks in hospitals, it has become clear that water supplies, vegetables, and flowers (which in turn might acquire the organisms from water and soil) entering hospitals are significant sources of the organisms. Thus, there is developing a concern for the ecology of the species outside of hospitals.

Investigations have been undertaken which indicate that sewage is a major source of P. aeruginosa in the environment, although the bacteria can grow in enriched surface waters at elevated temperatures. Furthermore, the bacteria are removed effectively during treatment of sewage. Nothing is known of the survival of P. aeruginosa during disinfection of wastes and regrowth. Investigations of hospital wastes indicate that while counts of indicater bacteria are often significantly higher in municipal sewage than in hospital waste, counts of P. aeruginosa, so common in hospital environments and resistant to disinfection, may be much higher in hospital sewage. Grabow and Nupen (Water Res. 6:1557-1563, 1972) have warned against the discharge of primary treated hospital effluents into streams.

Antibiotic resistant E.coli. Antibiotic resistance develops in bacterial populations as a result of the selective pressure of antibiotic usage. Resistance frequently develops in populations of bacteria when antibiotics are employed in therapy or as supplements in animal feeds. Resistance develops not only to the antibiotics applied, however, but to as many as 7 additional antibiotics as well. The genes conferring resistance are carried by an extra-chromosomal infectious particle which can be transferred among the Enterobacteriaceae, P. aeruginosa, and V. cholerae as well as several other bacterial species outside the Enterobacteriaceae.

The significance of infectious antibiotic resistance able to pass from species to species and from genus to genus is evident, and Grabow, Prozesky, and Smith (Water Res. 8:1-9, 1974) have called for a re-evaluation of bacterial water quality criteria since water may play an important role in the spread of coliform and other bacteria carrying antibiotic resistance transfer factors. Survival of coliforms carrying transferable resistance has been demonstrated in many swimming waters and sea water, and several studies have indicated that sewage discharges may contain substantial numbers of resistant organisms. This is especially true of hospital wastes (and probably animal wastes as well). A large proportion of coliform bacteria in hospital wastes carries transferable resistance, and the proportion does not appear to diminish during biological treatment. No studies have been conducted to determine the survival of resistant coliforms during chlorination. Drug resistant E.coli I do survive passage through stabilization ponds at least as well as do non-resistant strains, however.

OBJECTIVES OF STUDY

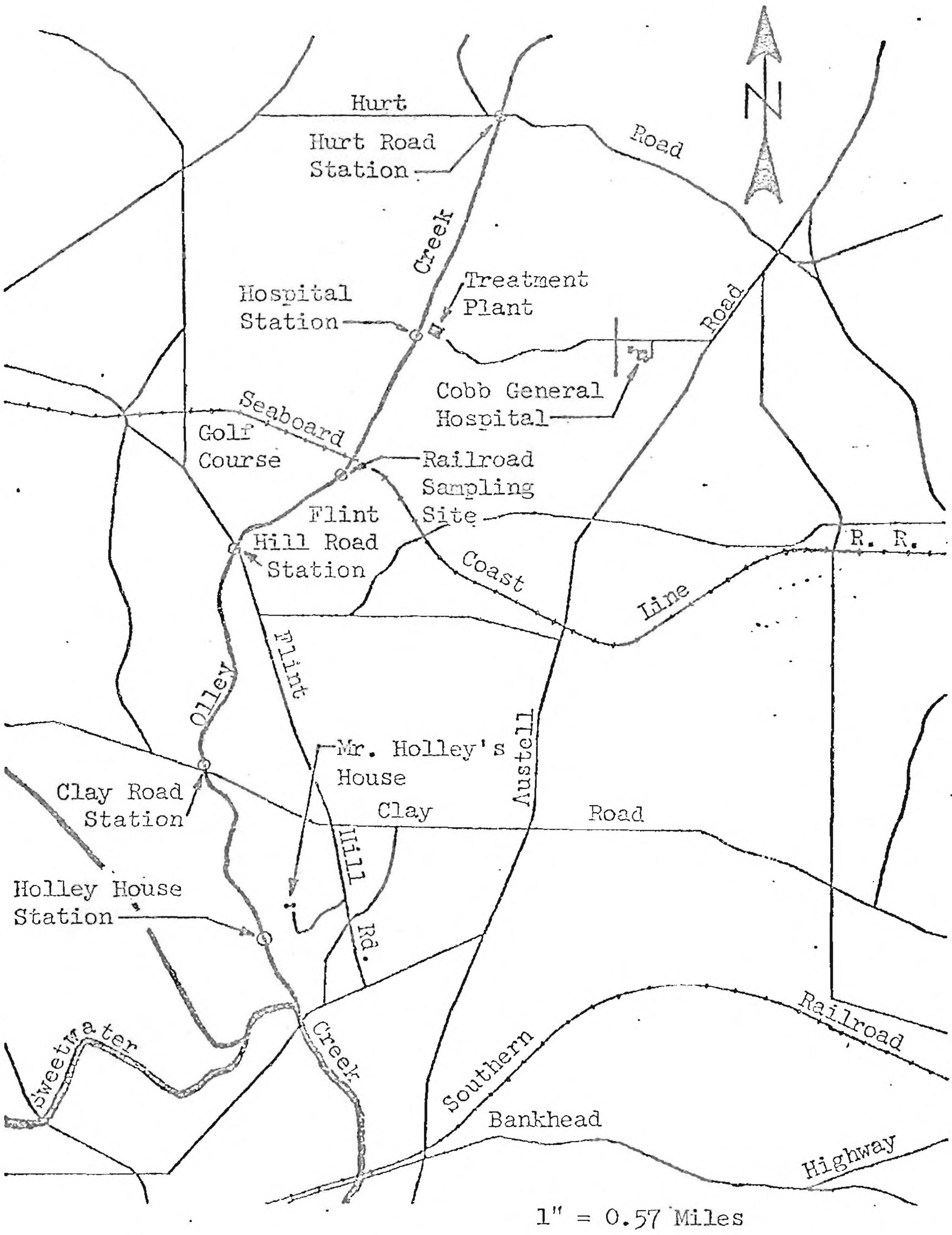
The Cobb General Sewage Treatment Plant treats the combined flow of sewage from the Cobb General Hospital and surrounding subdivisions. The treated wastes from the plant are discharged into Olley Creek which flows through a cattle farm and suburban residential land on its way to its confluence with Sweetwater Creek, which in turn serves as a drinking water supply for East Point, Georgia. In view of the exposure of human and animal populations to the receiving stream, the stream must be considered a potential reservoir of pathogenic bacteria and drug resistant bacteria.

The present study was undertaken to evaluate the effectiveness with which potentially harmful bacteria are removed during treatment at the Cobb General Sewage Treatment Plant.

THE STUDY SITE

The present studies were undertaken at the Cobb General Sewage Treatment Plant and Olley Creek into which the effluent from the treatment plant discharges. Olley Creek, a tributary of Sweetwater Creek (which is itself a tributary of the Chattahoochee River), is a small stream which flows from north to south in Cobb County near Austell, Georgia (Fig. 1). Approximately 0.8 miles

Figure 1. Location of stream sampling points



below the Hurt Road bridge over Olley Creek, the effluent from the Cobb General Waste Treatment Plant enters the Creek.

The Cobb General Waste Treatment Plant receives approximately 250,000 gpd of sewage, approximately 70,000 gpd of which is contributed by Cobb General Hospital (191 beds). During the present study, the flow entering the treatment plant was divided immediately so that half the flow by-passed the aeration chamber, clarifier, chlorinator, and baffles (Fig. 2). The by-passed flow was combined with the treated wastes prior to their discharge to a polishing pond which preceded release of wastes to Olley Creek. Division of the flow was undertaken because of periodic failure to maintain the sludge, probably because of slugs of toxic disinfectants from the hospital and washout, when the total flow passed through the plant which was designed for a flow of 125,000 gpd.

The portion of the raw waste being treated prior to discharge to the polishing pond, entered a package aeration plant haveing a detention time of 10 to 12 hours in the aeration basin and about 3 hours in the clarifier compartments. The overflow from the clarifier compartments entered a chlorination compartment which was followed by a baffle chamber providing from 5 to 10 minutes contact. The chlorinator was not functional during the study until early in the morning of August 23, several hours prior to obtaining the final samples.

The combined treated and by-passed wastes entered the polishing pond prior to discharge to Olley Creek. The pond is 3 feet deep with a theoretical detention time of 6.8 days when the flow is 250,000 gpd.

Olley Creek flows south for a distance of approximately 2.8 miles to its confluence with Sweetwater Creek. The creek flows through land which varies from thick undergrowth and swampy mud flats to meadows. Immediately downstream from the discharge is a cattle raising operation on which the cattle have easy

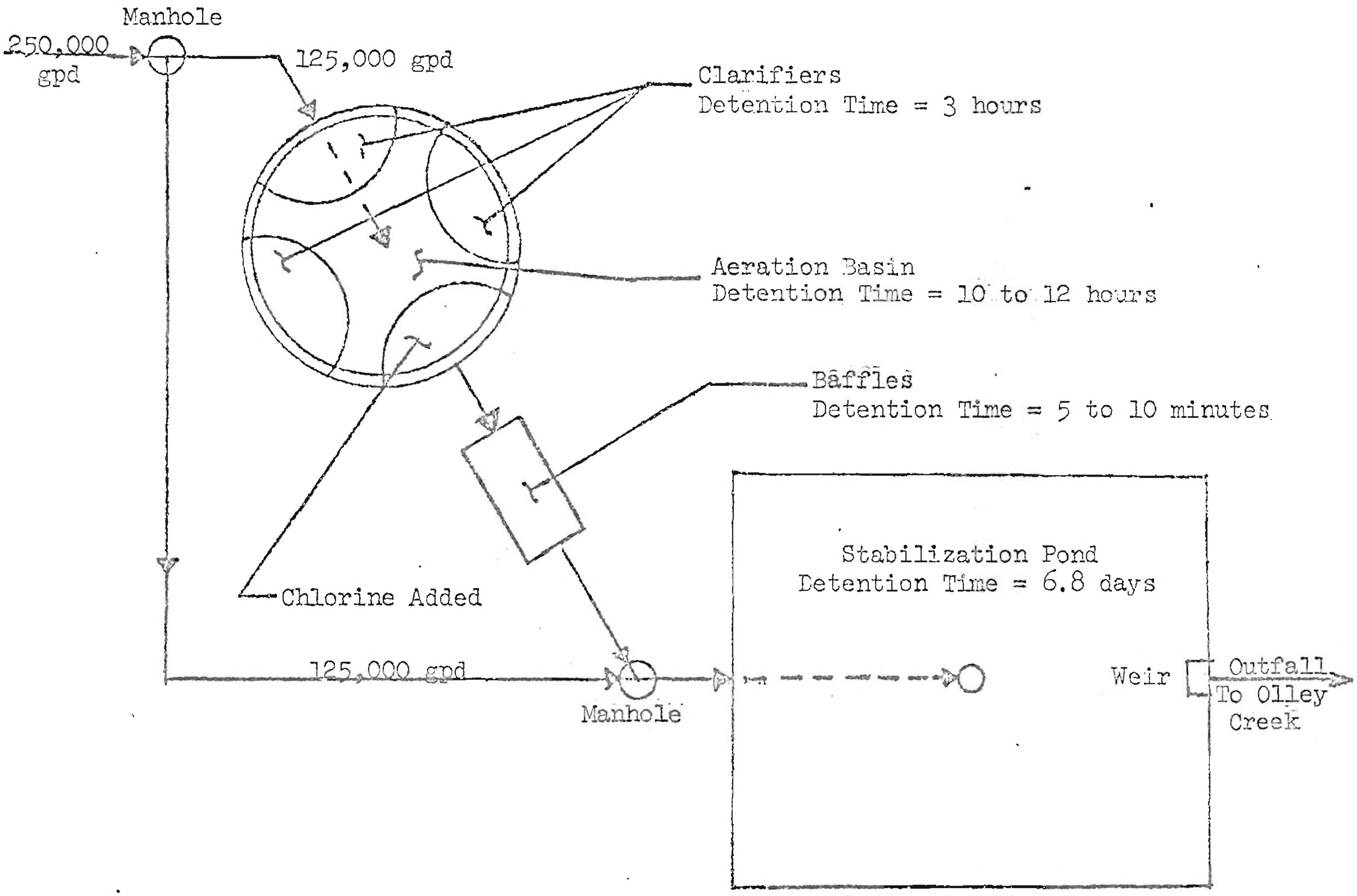


Figure 2. The Cobb General Wastewater Treatment Plant

access to the stream. Because the stream is known by residents of the area to be polluted, however, it is fenced off by many residents of adjacent land to reduce accessibility to cattle and children. However, household pets are not prevented from coming into contact with the water of the stream. After it passes through the cattle farm, the stream winds through a golf course, and then through sparcely populated areas, passing near to private dwellings in several places as it flows to Sweetwater Creek.

Sampling points were located at the Hurt Road Bridge unstream from the waste discharge, about 100 feet downstream from the waste discharge, at a railroad trestle near the golf course, at the Flint Hill Road Bridge, at the Clay Road Bridge, and near the Holley House (Fig. 1). The station at the Hurt Road Bridge was located about 0.8 miles upstream from the outfall. Located just upstream from this station was a manhold from which wastes leaked following heavy rains. The station at the Cobb General Waste Treatment Plant was accessible via a cow pasture and was located at a cattle crossing. Distances from the outfall to stations located at road crossings below the waste outfall are shown in Table 1. The station at the Holley House was located approximately 2.45 miles downstream from the outfall.

Flow measurements were obtained on July 9, 1974, and are presented in Table 1. Time of flow was calculated as the time between peak concentrations of Rhodamine B dye determined with a Turner fluorometer. Discharge was measured using a Price current meter and the procedure described by Linsley.*

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*Linsley, R. K., M. A. Kohler, and J. L. H. Paulhus. 1958. Hydrology for engineers. McGraw-Hill Book Co., New York.

Table 1. Flow characteristics at sampling stations

in Olley Creek, July 9, 1974

Sampling Station	Distance below discharge (mi.)	Time of flow (min) Between stations	Time of flow (min) Below discharge	Flow (cfs)	Dilution factor
Cobb General Hospital	0		0	5.53	1.0
		50			
Railroad Trestle	0.54		50	6.74	0.8204
		49			
Flint Hill Road	1.0		99	7.11	0.7778
		84			
Clay Road	1.85		183	8.89	0.6220

METHODS

Populations of *P. aeruginosa* and fecal coliforms were determined at the sampling stations on Olley Creek and through the Cobb General Waste Treatment Plant. Most probable numbers (MPNs) of *P. aeruginosa* were determined in the stream waters on April 13, April 20, April 27, and July 1. MPNs were determined according to procedures described by Hoadley et al. (Arch. Hyg. Bakteriol. 152:328-338, 1968). Populations of fecal coliforms in Olley Creek were determined on July 2. Counts of fecal coliforms were made in triplicate on membrane filters (MF) incubated on m-FC medium and 5 tube MPNs were determined, both according to *Standard Methods*. MPNs of *P. aeruginosa* and both MF counts and MPNs of fecal coliforms were determined at the Cobb General Waste Treatment Plant on July 17, July 26, and August 23, employing the procedures described above.

On July 26 and August 23, counts of total coliforms were made on MacConkey's agar and MacConkey's agar containing 10 µg/ml of ampicillin, 25 µg/ml of tetracycline, and 15 µg/ml of streptomycin to determine the populations of coliforms carrying drug resistance.

Furthermore, colonies of lactose fermentors growing on membrane filters in the fecal coliform test were isolated and were tested for their resistance to 12 antibiotics* employing the method of Bauer et al. (Amer. J. Clin. Pathol. 45:493-496, 1966). From the resulting data, frequencies of resistance to each antibiotic at each station and overall frequencies of multiple resistance were determined. Finally, 140 fecal coliforms colonies picked from membrane filters were examined using "Enterotubes" (Roche Diagnostics) to determine their identity.

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*Ampicillin, streptomycin, neomycin, cephalothin, polymyxin B, tetracycline, Kanamycin, nalidixic acid, chloramphenicol, colistin, streptomycin.

Resistance to 7 antibiotics** was determined on isolates of *P. aeruginosa* obtained on July 26 and August 23.

** Sentanycin, neomycin, streptomycin, tetracycline, carbenicillin, kanamycin, chloramphenicol.

RESULTS AND DISCUSSION

BACTERIAL POPULATIONS IN OLLEY CREEK

Populations of *P. aeruginosa* and fecal coliforms in Olley Creek are given in Tables 2 and 3. Most probable numbers of fluorescent pseudomonads able to grow at 39C (total) and *P. aeruginosa* were determined on 4 dates between April 13 and July 1. Populations of both groups at Hurt Road indicate that the stream was influenced by upstream waste sources and overflow from a manhole located adjacent to the stream. The higher populations occuring in the early spring reflect the lower water temperature, and consequent longer survival of the bacteria entering upstream, as well as overflow from the manhole following rain.

Populations immediately below the discharge from the Cobb General Waste Treatment Plant were generally lower than at the Railroad Trestle 0.54 miles downstream, probably because the waste was not mixed well with the stream water at this point. This became clear during dye tracer studies in which the dye was added to the stream at the discharge. During April, populations were high in the stream at the outfall and railroad trestle, resembling populations in raw sewage. The populations remained high throughout the reach of stream sampled, providing ample opportunity for transfer to animals and man having contact with the water. On July 1, when water temperatures were higher, the MPNs of *P. aeruginosa* decreased more rapidly, although populations of total fluorescent pseudomonads remained high, probably because they grew in the enriched stream water.

Counts of fecal coliforms in Olley Creek were determined on July 2. Counts were high and remained high as the water flowed downstream. Counts on July 2 were about 3 orders of magnitude lower than might be expected in raw sewage, as were counts of *P. aeruginosa* on the previous day.

Table 2. Most probable numbers of total fluorescent pseudomonads able to grow at 39C and *P.aeruginosa* in Olley Creek, Spring, 1974

Most probable number/100 ml

Sampling Station	April 13[1]		April 20[2]		April 27[3]		July 1[4]	
	Total	P.aeruginosa	Total	P.aeruginosa	Total	P.aeruginosa	Total	P.aeruginosa
Hurt Rd.	$> 1.6 \times 10^3$	9.2×10^2	9.2×10^2	9.2×10^2	2.0×10	2.0×10	1.6×10^2	5.0×10^0
Cobb General Hospital	1.3×10^4	9.2×10^3	2.2×10^3	2.2×10^3	$> 1.6 \times 10^5$	1.6×10^5	4.9×10^4	7.0×10^2
Railroad	$> 1.6 \times 10^5$	$> 1.6 \times 10^5$	4.9×10^4	3.5×10^4	8.0×10^2	8.0×10^2	3.3×10^3	5.0×10
Flint Hill Rd.	$> 1.6 \times 10^4$	$> 1.6 \times 10^4$	$> 1.6 \times 10^4$	$> 1.6 \times 10^4$	8.0×10^2	$< 2 \times 10^2$	2.3×10^3	1.4×10^2
Clay Rd.	$> 1.6 \times 10^3$	1.3×10^3	2.2×10^3	3.1×10^2	7.0×10^2	2.6×10^2	1.4×10^2	--
Holley House					1.7×10^2	3.3×10	1.3×10^3	

1. Temperatures 17-18C, overflow from manhole entered Creek at Hurt Road
2. Temperatures 18-21C
3. Temperatures 13-16C
4. Temperatures 24-26C

Table 3. Counts of fecal coliforms in

Olley Creek, July 2, 1974

Sampling Station	Count/100 ml
Cobb General Hospital	2.1×10^5
Railraod	4.0×10^4
Flint Hill Rd.	1.1×10^4
Clay Rd.	1.0×10^4
Holley House	1.1×10^4

The counts of both _P. aeruginosa_ and fecal coliforms, because they emanated from hospital wastes and because of their access to animal and human populations living adjacent to the stream, are of public health concern. The nature of these populations, and the effectiveness of the treatment process are therefore of concern.

REMOVAL OF BACTERIA DURING TREATMENT

Counts of _P. aeruginosa_, total coliforms, and fecal coliforms through the Cobb General Waste Treatment Plant were determined on 3 occasions: July 17, July 26, and August 23. The chlorinator was not in operation until the morning of August 23, however.

Populations of _P. aeruginosa_ (Table 4) in the raw wastes were typical of populations ordinarily found in raw sewage. Treatment in the activated sludge plant prior to discharge to the polishing pond resulted in reductions of from 97.5% to 99.8%. However, by-passing of flow resulted in a substantial reduction in the efficiency of removal prior to discharge to the pond. It is questionable whether passage through the pond benefited the treatment of the wastes as measured by the discharge of _P. aeruginosa_. The population of _P. aeruginosa_ in the pond effluent on July 17 was nearly identical to the population entering the pond. Although the population in the discharge from the pond on July 26 was less than the population in the activated sludge effluent, the population density in the discharge on August 23 was 90% of that in the raw waste, and equal to most raw sewage discharges. Furthermore, chlorination of the activated sludge effluent on August 23 appeared to have little effect on the population of _P. aeruginosa_, producing only about a 50% reduction in the MPN. The populations in the effluent to the stream were very high at all times.

Identical conclusions must be drawn from total and fecal coliforms populations in samples obtained on the same days (Tables 5-7). No significant

Table 4. Most probable numbers of *Pseudomonas aeruginosa* at the Cobb General Sewage Treatment Plant

Sampling Station	Most probable number/100 ml July 17, 1974	July 26, 1974	Aug. 23, 1974
Influent	2.2×10^5	2.6×10^5	1.3×10^6
Activated Sludge Effluent	5.4×10^3	5.0×10^3	2.7×10^3
Chlorinated Effluent			1.4×10^3
Effluent to Pond (Raw and Treated)	5.4×10^4		
Pond (Stagnant edge)	2.1×10^3	5.0×10^3	2.3×10^4
Effluent from Pond	5.0×10^4	1.7×10^3	1.3×10^5
Olley Creek	9.1×10^2		

Table 5 . Populations of Coliforms at the Cobb General Sewage Treatment Plant, July 17, 1974

Station	Coliforms/100 ml		
	MPN /100 ml		MF
	Presumptive	Fecal	Fecal
Influent	2.4×10^7	2.4×10^7	7×10^6
A. S. Effl.	1.7×10^5	1.7×10^5	1.5×10^5
Effl. to Pond (Raw + Treated)	$> 2.4 \times 10^7$	1.6×10^7	7.7×10^6
Pond (Stag. End)	4.9×10^6	4.9×10^6	2.1×10^6
Effl. from Pond to Stream	1.7×10^7	1.1×10^7	7.0×10^6
Olley Creek	$> 2.4 \times 10^6$	$> 2.4 \times 10^6$	2.9×10^6

Table 6 . Populations of Coliforms and Antibiotic Resistant Coliforms at the Cobb General Sewage Treatment Plant, July 26, 1974

Station	Coliforms/100 ml			Counts on MacConkey's AGAR						
	MPN		MF		Ampicillin		Tetracycline		Streptomycin	
	Presumptive	Fecal	Fecal	Total/100	No./100 ml	%	No./100 ml	%	No./100 ml	%
Influent	3.3×10^7	2.4×10^7	$1. \times 10^7$	2.4×10^7	2.9×10^7	[120.8][1]	4.6×10^6	19.2	2.3×10^7	95.8
A.S. Effluent	1.7×10^5	7.9×10^4	1.1×10^5	3.6×10^5	1.3×10^5	36.1	(2×10^4)[2]	(5.6)	9.3×10^4	25.8
Pond (Stag. Edge)	7.9×10^6	3.5×10^6	2.4×10^6	(5.6×10^6)	(7×10^6)	[177.7]	---	--	---	--
Effl. From Pond	1.7×10^7	1.7×10^7	1.4×10^7	1.8×10^7	1.4×10^7	77.8	(1.7×10^6)	(9.4)	4.3×10^6	(23.9)

1 Percentages in brackets in excess of 100%. Essentially all fecal coliforms resistant.
2 Numbers in parentheses were based upon low counts on plates

Table 7 . Populations of Coliforms and Antibiotic Resistant Coliforms at the Cobb General Sewage Treatment Plant August, 23, 1974.

	Coliforms/100 ml			Counts on MacConkey's AGAR						
	MPN		MFC		Ampicillin		Streptomycin		Tetracycline	
Station	Presumptive	Fecal	Fecal	Total/100 ml	No./100 ml	%	No.100/ml	%	No./100 ml	%
Influent	5.4×10^7	5.4×10^6	3.2×10^6	2.76×10^7	4.6×10^6	16.7	2.3×10^6	8.4	1.0×10^6	3.6
Act. Sludge Effluent	7.9×10^5	2.2×10^5	3.5×10^5	5.4×10^5	4.0×10^4	7.4	6.3×10^4	11.9	1.0×10^4	1.9
Chlorinated Effluent	1.6×10^5	9.2×10^4	4.5×10^4	3.83×10^5	1.3×10^5	33.9	1.3×10^4	3.4	2.5×10^4	6.5
Eff. to Pond (Raw & Treated)	3.5×10^6	2.7×10^6	1.73×10^5	2.4×10^6	1.0×10^6	41.6	1.3×10^5	5.4	--	--
Stagnant Edge	2.4×10^7	1.6×10^7	5.3×10^6	6.7×10^6	5.0×10^6	74.7	--	-	5.5×10^5	8.2
Eff. from Pond	9.2×10^6	9.2×10^6	6.8×10^6	7.03×10^6	2.5×10^6	35.6	--	-	5.0×10^5	7.1

differences existed between populations of fecal coliforms or total coliforms in the raw wastes and the discharge to the stream from the polishing pond on ony of the three sampling dates, in spite of removals in excess of 98% in the activated sludge portion of the plant. Chlorination of the activated sludge effluent on August 23 provided little better than a 50% reduction in the MPN of fecal coliforms, although an apparent reduction of 92% was suggested by counts on membrane filters. The MPN probably provided a more realistic estimate of the reduction, however, since cells injured, but not killed are known not to recover on membrane filters (Braswell and Hoadley, Appl. Microbiol. 28:328-329, 1974).

Whereas populations of *P. aeruginosa* were reduced during passage through the treatment plant, populations of fecal coliforms were not, and the value of the polishing pond again must be questioned seriously, as must the value of the plant as a whole if flow of raw waste is to be shunted around the aeration basin.

RESISTANCE OF ISOLATED BACTERIA TO ANTIBIOTICS

Resistance of bacteria to the action of antibiotics is a subject of considerable emerging concern especially since resistance is often transferable. That is, a resistant strain of *E.coli* often can transfer its resistance to a pathogen. If the *E.coli* and the pathogen reside in a person receiving antibiotic therapy, only the resistant cells survive, and a problem is created. The question today is whether exposure to reservoirs of resistant bacteria, especially those able to transfer their resistance, should be substantially reduced. Furthermore, pathogens can carry resistance to antibiotics, making control of infection difficult.

Psuedomonas aeruginosa is a pathogen of man and animals which can present a particular problem because it is resistant to most antibiotics. Isolates obtained during the studies of Olley Creek and the Cobb General Waste Treatment

Plant were examined for their resistance to 7 antibiotics. Normally most strains are resistant to chloramphenicol, kanamycin, neomycin, streptomycin, and tetracycline, but sensitive to gentamicin and carbenicillin which are administered intravenously in hospitals, including Cobb General Hospital.

Until 1973, there existed in the literature no reports of resistance of P. aeruginosa to gentamicin and carbenicillin. However, particularly on leukemia wards, reports of resistant strains began to appear in that year. During the present investigations no strains were isolated which were resistant to gentamicin. However, strains resistant to carbenicillin were isolated from the stream below the waste discharge and from the wastes. No strains resistant to carbenicillin were isolated from the stream above the waste discharge (Table 8). The potential problem presented by the escape of P. aeruginosa resistant to carbenicillin, or indeed, any P. aeruginosa strain, is that it will find its way back to patients in hospitals by way of the carriers in the general population or agricultural products such as lettuce or tomatoes. Furthermore, the health of farm animals, especially cows and calves, can be placed in danger by exposure to such reservoirs. If reservoirs of P. aeruginosa, and especially resistant strains, exist, the chances of this happening are increased. Thus, the control of this reservoir represents a desirable goal.

While no studies of transferability of resistance from resistant coliforms were undertaken during the present investigations, resistance to 12 antibiotics was determined. Of 136 isolates examined, 95.6% were resistant to at least 1 antibiotic and 92.7% were resistant to al least 2 antibiotics (Table 9). These represent high proportions of the population resistant to antibiotics. Furthermore, while rare, resistance to as many as 10 antibiotics was encountered. This observation is exceptional. Bases upon studies undertaken at other hospitals, at least 60% of resistant strains should be able to transfer resistance. No significant changes in the frequency of strains resistant

Table 8. Resistance of *P. aeruginosa* Isolates from Cobb General Waste Treatment Plant and Olley Creek to carbenicillin

Source of Isolates	No. Tested	No. Resistant	% Resistant
Olley Cr., Above Outfall[1]	35	0	0
Olley Cr., Below Outfall[1]	186	24	12.0
Cobb General WTP[2]	109	20	18.4

1. Isolates obtained on April 13,20,27 and July 1

2. Isolates obtained on July 26 and August 23

Table 9. Frequency of multiple resistance to antibiotics among fecal coliforms isolated from the Cobb General Sewage Treatment Plant on August 23, 1974

Resistance to:	Influent (23)		Activated Sludge Effluent (19)		Chlorinated Effluent (29)		Effluent to Pond (24)		Stagnant Edge of Pond (13)		Effluent from Pond (28)		Total (136)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
10 antibiotics	1	4.3	0		0		0		0		0		1	0.7
9 "	0		0		0		1	4.2	0		0		1	0.7
8 "	1	4.3	1	5.3	1	3.4	0		0		1	3.6	4	3.0
7 "	0		0		3	10.3	2	8.3	0		4	14.2	9	6.6
6 "	5	21.9	4	21.0	1	3.4	0		0		2	7.2	12	9.6
5 "	2	8.6	2	10.6	4	13.9	7	29.1	1	7.7	3	10.7	19	13.9
4 "	3	12.9	4	21.0	5	17.3	5	20.8	1	7.7	2	7.2	20	14.6
3 "	1	4.3	6	31.5	3	10.3	6	25.0	0		5	18.0	21	15.3
2 "	9	39.4	2	10.6	7	24.1	3	12.4	8	61.5	4	14.2	33	24.0
1 "	1	4.3	0		4	13.9	0		2	15.4	3	10.7	10	7.3
0 "	0		0		1	3.4	0		1	7.7	4	14.2	6	4.4

to multiple antibiotics as the waste passed through the treatment plant, although there was exhibited a tendency towards resistance to fewer antibiotics.

Frequencies of resistance to different antibiotics among fecal coliforms isolated at the Cobb General Waste Treatment Plant on August 23 are presented in Table 10. The most commonly encountered resistance was to Ampicillin (Am) which occurred in 58.8% of all isolates, but 87% of isolates from the raw waste. Resistances to sulfathiazole (S), neomycin (N), cephalothin (CF), and polymyxin B (PB) also were encountered frequently. Resistance to all antibiotics save gentamicin was encountered. Differences between resistances of isolates in the raw waste and in the pond effluent cannot be regarded as significant. While the predominant resistance characteristics differ somewhat from the predominant characteristics observed at other hospitals, the general nature of the populations was similar. Again, while transfer experiments were not undertaken, it may be expected that well over half of the isolates were able to transfer resistance to at least some of the antibiotics.

IDENTITY AND RESISTANCE OF FECAL COLIFORMS ISOLATES

One hundred and forty bacterial isolates from plates of mFC medium (fecal coliforms) were examined using Enterotubes (Roche Diagnostics, Nutley, New Jersey). Less than 40% of the isolates were identified as *E.coli* (Table 11), whereas over 40% were identified as *Klebsiella* spp. and 19% were identified as *Enterobacter cloacae*. While fecal coliforms normally would consist mainly of *E.coli*, the hospital waste appears to contribute other species which may be of some direct concern, i.e. *Klebsiella* spp., especially in view of their origin. It is becoming clear nationally also, that *Klebsiella* spp. may at times be included in the fecal coliform group. Their significance, though, must be considered equal to that of *E.coli*.

Table 10. Frequency of resistance to 11 antibiotics among fecal coliforms isolated from the Cobb General Sewage Treatment Plant on August 23, 1974

	Number of Isolates	Resistance to Am		S		N		CF		PB		Te		K		NA		C		CL		St	
		No. R	% R	No. R	% R	No. R	% R	No. R.	% R	No. R	% R	No. R	% R	No. R	% R	No. R	% R	No. R	% R	No. R	% R	No. R	% R
Influent	23	20	86.9	10	43.5	9	39.1	11	47.8	6	26.1	6	26.1	8	34.8	4	17.4	7	30.4	7	30.4	4	18.2*
Eff. from Act. Sludge	19	12	63.2	13	68.4	11	57.9	8	42.1	9	47.4	9	47.4	8	42.1	4	21.1	4	21.1	0		2	10.5
Eff. from Chlor.	29	16	55.2	13	44.8	12	41.4	9	31.0	24	82.8	12	41.4	6	20.7	4	13.8	2	6.9	2	6.9	2	6.9
Eff to pond (Raw & Treated)	24	10	41.7	14	58.3	13	54.2	14	58.3	19	79.2	10	41.7	4	16.7	5	20.8	4	16.7	4	16.7	5	20.8
Stagnant edge of pond	13	3	23.1	7	53.9	6	46.2	5	38.5	1	7.7	1	7.7	2	15.4	1	7.7	0		1	7.7	0	-
Eff. from pond	28	19	67.9	14	50.0	13	46.4	15	53.6	2	7.1	11	39.3	8	28.6	7	25.0	4	14.3	3	10.7	1	3.6
Total	136	80	58.8	71	52.2	64	47.1	62	45.6	61	44.9	49	36.0	36	26.5	25	18.4	21	15.4	17	12.5	14	10.4

* % based upon 22 cultures tested

Table 11. Identification of 140 fecal coliforms isolated at the Cobb General Waste Treatment Plant, August 23, 1974

ORGANISM	NUMBER of Isolates	% of Isolates
Escherichia coli	54	38.6
Klebsiella spp.	58	41.4
Enterobacter cloacae	27	19.3
Enterobacter hafnia	1	0.7

The patterns of antibiotic resistance among the different fecal coliforms is of some interest (Table 12). It is clear that while about 90.4% of E.coli isolates were resistant to at least 1 antibiotic, and 80.8% were resistant to at least 2 antibiotics, 96.15% of Klebsiella isolates were resistant to at least 1 antibiotic, and 92.3% were resistant to at least 2. Furthermore, Klebsiella isolates tended to be resistant to many more antibiotics than were E.coli. Enterobacter cloacae isolates, while exhibiting less total resistance than Klebsiella and more total resistance than E.coli, exhibited less multiple resistance to 3 or more antibiotics than did isolates belonging to the other groups.

The frequencies of resistance to individual antibiotics also differed in several respects. With the exception of resistance to cephalothin and polymyxin B, a larger proportion of Klebsiella isolates than E.coli isolates were resistant to any given antibiotic. Only resistance to cephalothin was significantly more frequent among E.coli than Klebsiella. The frequencies of resistance to several antibiotics among Klebsiella isolates were significantly higher than among E.coli isolates. These included ampicillin (94.3% vs. 25.0%), sulfathiazole (77.0% vs. 38.5%), tetracycline (55.8% vs. 15.4%), and nalidixic acid (30.7% vs. 9.6%). The more frequent occurrence of resistance may reflect treatment of Klebsiella infections with antibiotics, resulting in a higher rate of exposure of Klebsiella than E.coli to antibiotics. This is of importance when evaluating the significance of coliform populations in the stream since the Klebsiella populations are themselves of public health concern.

Table 12. Frequency of multiple resistance to antibiotics among fecal coliforms subgroups isolated from the Cobb General Sewage Treatment Plant on August 23, 1974.

Resistance to:	*E.coli* (52)	*Klebsiella* (52)	*Enterobacter cloacae* (27)
10 antibiotics	0	1 (1.93)*	0
9 antibiotics	0	0	1 (3.7)
8 antibiotics	2 (3.85)	1 (1.93)	1 (3.7)
7 antibiotics	1 (1.93)	7 (17.46)	1 (3.7)
6 antibiotics	1 (1.93)	8 (15.4)	2 (7.4)
5 antibiotics	7 (13.46)	12 (23.1)	0
4 antibiotics	9 (17.3)	9 (17.3)	2 (7.4)
3 antibiotics	8 (15.4)	8 (15.4)	4 (14.8)
2 antibiotics	14 (26.9)	4 (7.7)	12 (44.5)
1 antibiotics	5 (9.62)	2 (3.85)	3 (11.1)
0 antibiotics	5 (9.62)	0	1 (3.7)

*Percentage of isolates in parentheses.

Table 13. Frequency of resistance to 12 antibiotics among fecal coliform subgroups isolated from the Cobb General Sewage Treatment Plant on August 23, 1974.

RESISTANCE TO:	*E. coli* (52)	KLEBSIELLA (52)	*ENTEROBACTER cloacae* (27)
AMPICILLIN	13 (25.0)*	49 (94.3)	16 (59.3)
SULFATHIAZOLE	20 (38.5)	40 (77.0)	10 (37.0)
NEOMYCIN	25 (48.1)	30 (57.7)	6 (22.2)
CEPHALOTHIN	29 (55.8)	11 (21.2)	20 (74.1)
POLYMYXIN B	29 (55.8)	24 (46.2)	6 (22.2)
TETRACYCLINE	8 (15.4)	29 (55.8)	11 (40.8)
KANAMYCIN	11 (21.2)	19 (36.6)	4 (14.8)
NALIDIXIC ACID	5 (9.6)	16 (30.7)	4 (14.8)
CHLORAMPHENICOL	5 (9.6)	12 (23.1)	3 (11.1)
COLISTIN	6 (11.5)	8 (15.4)	2 (7.4)
STREPTOMYCIN	6 (11.5)	7 (13.5)	1 (3.7)
GENTAMYCIN	0	0	0

*Percentage of isolates in parentheses.

CONCLUSIONS

The following conclusions were drawn from the results of the present study:

1. Populations of both fecal coliforms and *P. aeruginosa* in Olley Creek were high, reflecting a high level of fecal pollution originating from the Cobb General Waste Treatment Plant.

2. The populations of *P. aeruginosa* and fecal coliforms in Olley Creek represented a potential threat to the public health in view of contact of cattle with the stream waters and the accessibility of the stream which passes through a golf course and near private homes. *Pseudomonas aeruginosa* and klebsiellas included among fecal coliforms may cause disease and indicate that other pathogens probably are present as well.

3. Populations of fecal coliforms were not reduced during passage of wastes through the treatment plant. In terms of the removal of this group of indicator bacteria, the treatment plant played no functional part.

4. Populations of *P. aeruginosa* were reduced only by about 90% by treatment. This reduction cannot be considered significant in view of the large populations in the final effluent.

5. By-passing of wastes as it was practiced at the treatment plant contradicted, from a bacteriological point of view, what little was accomplished during aeration of those wastes passing through the package plant.

6. The occurrence of *P. aeruginosa* isolates resistant to carbenicillin in the stream below the effluent must be considered undesirable since such organisms are not normally encountered. Resistance ordinarily develops only following prolonged therapy, and the existance of a reservoir of such resistant strains in the environment may pose a threat to immunologically compromised persons and animals.

7. The high levels of multiple resistance to antibiotics among fecal coliforms likewise must be viewed with concern, since they find their way into the human and animal populations where, potentially, transfer of resistance to pathogens may occur.

RECOMMENDATIONS

It is considered that a potentially hazardous waste of the kind described, emanating from a hospital, and bearing pathogens and antibiotic resistant bacteria, should be treated to a high degree and rendered harmless prior to discharge to a watercourse, especially where human and animal contact with the receiving stream is likely. Such protection of the environment of man is not provided at Olley Creek.

It is recommended that prior to discharge to the stream, the final effluent from the Cobb General Waste Treatment Plant be chlorinated to such a degree that both _P. aeruginosa_ and fecal coliform populations are reduced to a low level. Chlorination equipment must be dependable and kept in good repair. Some experimentation will be necessary to assure proper levels of chlorination. Whereas short circuiting in the polishing pond is probable, the effluent contains algae which interfere with the efficacy of chlorination. It is possible that the pond, as it is presently operated, causes more harm than it does good from a bacteriological point of view.